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THE EFFECT OF RADIUM RADIATIONS ON THE DEVELOPMENT OF CHÆTOPTERUS.

CHARLES PACKARD.

INTRODUCTION.

The experiments described in this paper show that the egg of *Chætopterus*, if radiated for an appropriate length of time before insemination will be injured to such an extent that its nucleus plays no part in development. Nevertheless the sperm nucleus divides normally; development is therefore androgenetic. The history of the egg nucleus from the time of insemination until it is absorbed into the protoplasm can be followed in detail.

Similar experiments have been performed by Gunther Hertwig ('11) on the frog's egg. He observed that if the eggs, after a brief radiation, were inseminated with fresh frog sperm, the amount of injury appearing in the embryos is proportional to the length of exposure. But if the eggs are radiated for more prolonged periods, cleavage becomes fairly normal, and the embryos, somewhat retarded in their rate of development, grow into swimming larvæ. These larvæ are normal in shape but are smaller than the controls and not as active, in these respects resembling parthenogenetic larvæ. He also observed that the nuclei of the cells in the embryos developing from eggs radiated for considerable periods are about one half of the size of the nuclei found in the controls. Since the size of the nucleus, according to Boveri, varies with the number of chromosomes contained in it, the inference is that these embryos have developed under the influence of the sperm nucleus and are therefore androgenetic. The egg nucleus has been entirely prevented from taking part in development. Cytological proof of this critical point was lacking.

The experiments of Paula Hertwig ('16) on the eggs of *Triton* do not wholly confirm the results just described. Exposures of the eggs for 5 to 30 minutes to a preparation of 51 mg. of radium

bromide, previous to insemination, resulted in embryos which were both normal and abnormal. In general there was little difference between the results obtained from the short and from the long exposures. Measurements of the nuclei in the radiated embryos showed that they were approximately one half the normal size and the development was presumably androgenetic. The actual number of chromosomes could not be counted with certainty. Hertwig states that "a certain answer to this question (of the exact number of chromosomes) could be furnished only by a study of the eggs during their early development. Since this is impossible, owing to practical difficulties which the material presents, the question must remain unsolved."

In the reverse experiment, in which frog sperm was radiated, it was found by Gunther Hertwig ('11) that the same developmental conditions obtained which he found when the egg was radiated. Normal eggs fertilized by sperm which had been radiated for short periods gave rise to abnormal embryos. But if the sperm is radiated for a long period the embryos developed normally. Later he repeated this experiment on the sea urchin and found that although the intensely radiated spermatozoon can penetrate the normal egg it fails to develop within the egg, and brings about an abnormal type of cleavage. The egg nucleus may divide by itself, sometimes normally, but more often irregularly, as though the sperm had acted as a mechanical hindrance to orderly cleavage. When the sperm had not thus interfered in the first mitosis it usually fused with the nucleus of one of the blastomeres, thus causing abnormalities in the descendants of that cell. In all of these cases the sperm aster developed normally, the centrosome divided, and the daughter centrosomes became the centers of the first cleavage figure. This fact lead Hertwig to doubt Boveri's conception of the centrosome as a permanent cell organ, and to incline to Lillie's view that it is a result of the interaction of the sperm and the egg protoplasm.

MATERIAL AND METHODS.

The eggs of the tubicolous annelid, *Chætopterus pergamentaceus*, are ideal for the type of experiments described in this paper. They can be obtained fresh and in sufficient quantities,

polyspermy is rare, and the control eggs develop with perfect regularity if kept under appropriate conditions. The number of chromosomes is small, the haploid number being nine, and the achromatic structures are unusually large. The most valuable feature is the fact that the unfertilized eggs, when placed in sea water, proceed in their development as far as the metaphase of the first maturation division, at which time further development ceases. Now it has been shown (Packard, '16) that chromatin, when in its most condensed stage at the time of the metaphase, is particularly susceptible to radium radiations. A short exposure therefore is sufficient to injure the chromatin without harming the protoplasm. This is an important point, for I have shown (Packard, '15) that prolonged radiations injure the protoplasm and bring about a very abnormal type of development quite different from that which follows the injury of the chromatin alone. A further advantage possessed by these eggs is their extremely labile condition. As Miss Allyn ('12) showed, they can be stimulated to develop parthenogenetically by a great number of agents. I have found that prolonged exposures will not prevent it from developing when fertilized by a normal sperm.

The metaphase of the first maturation division in the egg of *Chaetopterus* is reached about twenty minutes after the unfertilized eggs are put in sea water. In carrying out an experiment I allowed the eggs to remain for this period in sea water and then exposed them to the radium. A quantity of eggs is gathered into a compact mass in the center of a watch glass containing a measured quantity of water. The tube containing the radium bromide, having the strength of 50 mg. of pure bromide, is now held over the center of the mass of eggs at a distance of $\frac{5}{16}$ inch above it. It is obvious that the eggs did not all receive exactly equal amounts of radiation, but the difference in amount which each received is very slight.

The alpha rays are not able to penetrate the thin walls of the glass tube in which the radium salt is held and are therefore not responsible for any of the effects obtained. Probably some of the slower beta rays are also cut off by the glass or by the sea water over the eggs; but the great majority of beta rays and all of the gamma rays are able to reach the eggs.

The unfertilized eggs were exposed for periods varying from 5 to 135 minutes, after which they were inseminated with *Chætopterus* sperm, and their behavior in the formation of polar bodies and in cleavage recorded. In all of these experiments controls were kept, made up from the same lot of eggs and subject to the same temperature conditions.

For cytological study the radiated eggs were killed, at various times after insemination, in Meves' fluid, Boveri's picro-acetic, and in Bouin's fluid. The last named reagent proved to be the best for most purposes. It acts rather destructively on the centrospheres, but is otherwise very good.

THE NORMAL DEVELOPMENT OF *Chætopterus*.

The process of maturation and fertilization in *Chætopterus* has been described by Mead ('97). When the eggs are taken from the genital segments of the female and put into sea water the wall of the germinal vesicle breaks down. Fibers from two well-developed egg asters grow in toward the chromosomes which gradually assume an equatorial position. The spindle thus established moves toward the animal pole, rotating as it goes, until it comes to rest at right angles to the periphery of the egg. The chromosomes are already split, being tetrads at this time, but no actual separation occurs until after the sperm has penetrated the egg. In this condition the egg remains until fertilized.

As soon as the sperm has penetrated the egg the first polar body is quickly extruded, about 15 minutes after insemination. The second polar body is given off about 10 minutes later. Each chromosome remaining within the egg bends into a V shape and the entire group of nine gathers about the remaining egg aster. Before the group begins to move inward toward the center of the egg each chromosome becomes vesicular. During the inward journey the separate vesicles fuse to form a single large egg nucleus. The egg aster disappears when the fusion of the chromosomal vesicles takes place.

The sperm head does not begin to grow until it has penetrated the egg for some distance. But before its growth commences a small sperm aster with a centrosome appears in front of it. As the sperm moves inward the centrosome divides, each daughter

centrosome being surrounded by a well-marked aster. Finally the sperm grows into a vesicular form resembling the egg vesicle with which it soon comes in contact. Actual mingling of the chromatin elements does not occur until the metaphase of the first cleavage. Up till that time the chromosomes from each nucleus can easily be distinguished. Before the chromosomes have condensed into their characteristic shape there can be seen in the cleavage nucleus a number of chromatin nucleoli, probably derived from the chromosomes. During cleavage these lag behind, staying in the cleavage plane. The achromatic structures are very prominent, the asters remaining even after the completion of cleavage.

OBSERVATIONS ON THE LIVING EGGS.

To test the effect which gradually increasing amounts of radiation will produce in the rate of cleavage and in the production of abnormal cleavages it is necessary to employ throughout any one experiment eggs taken from a single female, for it was found that eggs from different specimens varied in the rate of cleavage and in other ways. In each experiment a sufficient quantity of eggs was exposed, and from this number a few were taken off at 10-minute intervals and fertilized in finger bowls. The proportion of cleaving eggs, compared with the controls, and the number of abnormal cleavages were recorded. This experiment was repeated on different days, and with eggs derived from different females.

It was found that brief radiations do not affect the rate at which the polar bodies are extruded. Indeed, prolonged radiations have little effect, for only in a few instances could any unusual condition be found. A cytological study showed that as long a treatment as 80 minutes causes very few abnormal polar divisions. Cleavage is much retarded, the most pronounced retard being seen in eggs radiated for 30 to 40 minutes previous to insemination. Longer exposures result in a more normal rate. This unexpected result is due to the fact that eggs which have been radiated for a long time are almost all polyspermic and such eggs always divide rapidly, sometimes ahead of the controls, and always into three or more parts.

The significant feature in these experiments is the sudden drop in the rate of cleavage seen in eggs radiated for periods up to 40 minutes. The explanation for this phenomenon is to be found in the behavior of the egg nucleus during maturation and subsequent growth, and in the behavior of the sperm nucleus during cleavage.

CYTOLOGICAL OBSERVATIONS.

A cytological examination of eggs exposed for periods of 15 to 35 minutes previous to insemination shows that the first effect of the radium radiations is on the chromatin; the protoplasm is not visibly affected. The abnormal behavior of the egg nucleus is perfectly apparent, but even prolonged radiation produces no visible change in the protoplasm. Without doubt the latter is affected, for polyspermy becomes more and more common as the duration of the exposure is lengthened. In *Nereis*, polyspermy under these conditions is due to a weakening of the egg membrane and perhaps to a partial liquefaction of the protoplasm. The same explanation may hold true for *Chætopterus* also.

All the eggs exposed for periods up to 35 minutes show the same types of abnormalities but in varying degrees, the more pronounced being found naturally in eggs exposed for the longer periods. The outstanding feature in the development of these eggs is the ever increasing tendency of the germ nuclei to remain apart. This is not due to any unusual development of the sperm; its centrosome divides normally, the asters appear, and the whole apparatus moves inward at the usual rate (Fig. 1). But the egg nucleus often remains close under the region of the polar bodies and may even be connected with them by a protoplasmic bridge in which chromatin threads can be distinguished. This phenomenon grows more common as the duration of the exposure is increased to 35 minutes. In this position it develops more or less normally, depending on the length of exposure. During cleavage the egg chromatin always remains separate from the dividing sperm chromosomes and can easily be distinguished from them because of its position and appearance. With this brief statement of the general course of development in the radiated eggs we may now examine in detail some of the conditions found during the various stages of maturation and cleavage.

After the sperm enters, the first maturation is completed normally and the chromosomes become arranged on the second polar spindle. There are very few exceptions to this, even in eggs exposed for a long time. Fig. 2 shows a condition which may be found occasionally in eggs treated for 80 minutes or more. It is evident that the whole egg, both chromatin and protoplasm, has been greatly disturbed, for the sperm after entrance has failed to develop normally. In the course of normal development it grows into a large vesicle by the time it has reached the vicinity of the egg nucleus, but in this case there is no evidence of any such growth. The tripolar spindle of the first maturation division indicates again how extensive is the injury to the egg. The extra aster may be derived from the sperm aster, or possibly both sperm asters are involved, one having fused with the egg aster; or the latter may have divided, while the sperm aster has not developed at all. The latter view is very likely correct for in other eggs similarly treated the sperm may be seen lying in the center of the egg without any trace of centrosome or asters. Whatever may be the right interpretation it is evident that the egg protoplasm has been injured to such an extent that the sperm cannot develop normally. The same phenomenon can be seen in the eggs of *Nereis* similarly treated. The chromosomes on the tripolar spindle show no signs of fragmentation but they have not divided, and some of them are irregular in shape. But in eggs treated up to 45 minutes the formation of the polar bodies and the development of the sperm is practically normal. The egg nucleus in a fairly large proportion of cases develops normally after the extrusion of the polar bodies and fuses with the sperm nucleus. The subsequent development of such eggs will be described later.

In a considerable number of eggs exposed for periods up to 45 minutes the egg nucleus does not behave normally. Instead of moving inward to meet the advancing sperm, it tends to remain in its original position under the polar bodies, there developing into the vesicular stage. Fig. 3 illustrates this condition. Here, although a part of the nucleus has moved inward, there still remains a part of it directly under the polar bodies, connected with the rest by a protoplasmic bridge. The distribution of the chro-

matin follows the shape of the nuclear vesicle; a little is still in the polar position. This condition prevents the egg nucleus from flattening out against the sperm nucleus. The latter is seen normally developed with its astral rays extending far out into the protoplasm.

A more pronounced case of the same sort is seen in Fig. 4. Here the egg nucleus has hardly moved at all but has developed in approximately its original position. Its inner end has come under the influence of a sperm aster, and some of its chromatin is evidently polarized. Its outer end is attached to the second polar body by chromatin threads and the whole nucleus has the appearance of being anchored to it.

Modifications of this condition are seen in the polyspermic egg shown in Fig. 5. Most of the egg nucleus has migrated away from the pole but a small piece about the size of a single chromosomal vesicle is still attached to the second polar body by delicate strands. Two sperms have entered; one of them has entirely fused with the egg nucleus, and the other is about to fuse. Only three asters can be found in this case. In Fig. 6 two sperms have fused with the egg nucleus which, as in the preceding case, is still attached to the polar body. Five asters can be found in connection with this cleavage nucleus. The number of instances in which three or five asters appear make it evident that the original egg aster may persist.

A final stage showing how the egg nucleus in the vesicular condition may fail to fuse normally with the sperm nucleus appears in Fig. 7. The latter nucleus is about fully grown and its asters are well developed. The egg nucleus is developed only in part. The two small vesicles contain numerous chromatin granules. Between them the remainder of the egg chromatin lies free in the protoplasm in the form of rods and spheres. These masses are not chromosomes for they have not the typical size or appearance. The curious positions which they have assumed indicate that the force drawing them inward is not normal.

Those eggs which were radiated for periods up to 25 minutes do not show many of the abnormalities just described. In such eggs the pronuclei develop normally to all appearance and fuse with each other. The first indication that any injury has been

done to the egg chromatin appears at the anaphase of the first cleavage. Fig. 8 shows an egg in which this is the case. There has not been a complete mingling of the two nuclei for the egg chromosomes are left behind in the future cleavage plane. They are for the most part normal in appearance. One is stretched out to an unusual length as though pulled at each end by a spindle fiber. If this is the case, the spindle-fiber attachment is unusual for here it is evidently terminal while in the normal chromosomes it is median. The nine sperm chromosomes in each group show the characteristic V-shape. Division is thus regular and haploid, and the injured egg chromosomes have not interfered with the orderly division of the egg.

A more noticeable injury to the egg chromatin is seen in Fig. 9. Here again the nuclei probably fused normally to all appearances. But at the anaphase, when the sperm chromosomes are moving towards the poles, the egg chromatin is left behind in a very confused condition. A few chromosomes have formed but they are noticeably beaded as though on the point of breaking up into fine granules. The remainder of the chromatin is in irregular masses. Possibly its position outside of the spindle accounts for the fact that the chromosomes show little sign of being drawn towards the poles. No spindle fibers can be found attached to it.

When the nuclei have failed to fuse, as shown in Figs. 3 and 4, the further growth of the egg nucleus is greatly modified. In Fig. 10 the chromatin is seen to be condensed into rods and irregular masses which lie wholly outside of the mitotic spindle. The rod-shaped masses are beaded. Only a few of the chromosomal vesicles could have migrated inward.

A polyspermic egg in which the same type of abnormality is found is shown in Fig. 11. From the distribution of the chromatin it may be inferred that this condition is a further development of such an egg as is shown in Figs. 5 and 6. It is interesting to note that the egg chromatin which shows the greatest sign of injury remains nearest to the pole. The more normal chromatin has migrated inwards. The sperm chromosomes which are distributed very irregularly are of normal shape and size.

These observations show that when the unfertilized eggs are radiated for periods up to 30 minutes before insemination, the

nucleus develops normally and fuses with the sperm nucleus. But during cleavage the egg chromosomes do not move together with the sperm chromosomes but lag behind in the future cleavage plane. Under the influence of longer radiations the egg is so affected that its nucleus is unable to move from its position under the polar bodies, but develops there more or less abnormally, and plays no part in the cleavage of the egg. In both cases the cleavage is regular and haploid. A prolonged radiation so affects the egg that the polar bodies may be formed abnormally or not at all; in such instances the sperm also fails to develop, though it can move up to the position of the polar spindle. Polyspermy becomes more and more frequent as the duration of exposure increases. This leads to abnormal cleavage into three or more cells.

DISCUSSION.

The facts presented in the foregoing pages show that with increasing periods of exposure the number of diploid cleavages is lessened and the number of haploid cleavages, in which only the sperm nucleus takes part, is greatly augmented. Actual counts of sections of dividing eggs give a clear picture of the proportion which obtains between these two types. These counts are represented graphically in the accompanying text figure. The ordinates represent the percentage of eggs which cleave, and the abscissæ, the duration of the exposure in minutes. Polyspermic eggs were disregarded in making these counts because they belong to a different category of phenomena. The dotted line represents the percentage of eggs which divided regularly.

It is seen that brief exposures do not prevent eggs from dividing, but that exposures of 30 to 35 minutes bring about a marked decrease in the numbers which cleave. This relation was noticed in the living eggs, and the curve plotted from the latter data corresponds closely to that derived from a study of the sections. After this critical period the number of eggs which cleave regularly increases to about 70 per cent. and then, with prolonged exposures, gradually falls off until, after an exposure of 130 minutes, practically no eggs divide.

The other curves show why this sudden drop at the critical

period occurs. The number of eggs dividing with the full complement of chromosomes falls gradually up to the 30-minute period. Then it drops very suddenly so that after a treatment of 35 to 40 minutes very few eggs divide with the diploid number.

The proportion of eggs dividing wholly under the influence of the sperm rises rapidly at the critical period and remains high until prolonged radiation prevents cleavage altogether. Of the eggs which remain uncleft after the others have divided with either the diploid or the haploid number of chromosomes, most are in the condition shown in Fig. 9. These may divide at a much later time but I have no data on that point. It is evident, therefore, that during the critical period the egg nucleus has been injured to such an extent that it can take no part in cleavage, and in some way prevents or greatly retards the cleavage of the sperm nucleus. After that time it acts like a foreign body, exerting very little influence, if any at all.

The form of the curve shown in the dotted line of the text figure bears a striking resemblance to the curves figured by Hertwig ('11) in his article on the effect of radiations upon the unfertilized frog egg. In his experiments Hertwig exposed different lots of eggs to preparations of radium of different strengths, and then fertilized them with normal sperm. The criterion in estimating the effect of the treatment was the length of life of the embryos. In the three sets of experiments it appeared that with brief exposures the length of embryonic life varies inversely with the duration of the exposure. Thus, the eggs treated for 5 minutes before insemination with a preparation having the strength of 7.4 mg. of RaBr_2 lived for 5 days, at which age they were abnormal in many respects. An exposure of 15 minutes resulted in much greater injuries, and the embryos lived but 3 days. But with longer radiation, development was normal through the gastrula stage and the embryos lived 8 or 9 days. They were weak and small but fairly well formed. With weaker preparations of radium the results were less pronounced but of the same order.

Hertwig's explanation is that brief radiations injure the egg nucleus but do not prevent it from fusing with the sperm nucleus. However, the injury is sufficient to bring about the production of

abnormal nuclei in the later embryos, as he demonstrated cytologically. The longer exposures so injured the egg nucleus that it failed altogether to fuse with the sperm nucleus, and therefore played no part in later development. The embryos were therefore androgenetic.

He has brought forward no actual proof that this is the case in the frog egg, except the evidence furnished by measurements of the nuclei. Paula Hertwig has shown that when *Triton* eggs are similarly treated the number of chromosomes is less than the diploid number and probably the haploid number but of this she is not certain. But Hertwig's explanation is in part supported by the facts brought forward in this paper. A strict comparison cannot be made since his conclusions were based on the length of life of the embryos while mine have to do with the behavior of the germ nuclei up to the time of the first cleavage. But in all probability the same phenomena occur in both cases.

The results obtained in the reverse experiment, in which the frog sperm is first radiated and then added to fresh eggs, are of the same order as the foregoing. Up to a critical time the radiated sperm produces greater and greater abnormalities in the embryo; after that period, more intensely radiated sperm produces more normal embryos. The original experiment of O. Hertwig ('11) has been repeated by G. Hertwig ('12), Opperman ('13) and others with the same results. A cytological study of the eggs of the sea-urchin fertilized by radiated sperm has been made by G. Hertwig ('12). He found that after prolonged radiation the sperm retained their motility and entered the egg, but the sperm nucleus never increased to its normal size in the egg. As a rule it lay as a foreign body outside of the dividing egg nucleus, but occasionally it became involved in the spindle and the division was rendered abnormal, probably because of the mechanical interference. In such instances the sperm nucleus was carried passively into one of the blastomeres later fusing with the nucleus of that cell. The subsequent development of the cell and its descendants was abnormal. On the other hand, a shorter radiation injured the sperm nucleus but it fused with the egg nucleus. Later it was thrown out as in *Chætopterus*. In such instances the egg nucleus divided abnormally and cleavage

was irregular. Hertwig states that the abnormalities of the egg chromosomes are produced by the action of the injured sperm chromatin which operates like a poison. Its decomposition products in some way communicate the poison to the sound chromatin.

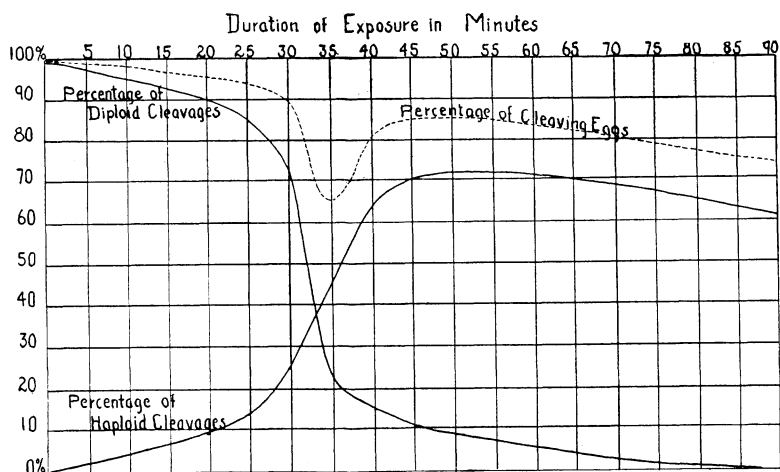
There is no evidence, other than that given by Hertwig, that radiated chromatin acts as a poison to the unirradiated nucleus. His figures in support of this idea are not convincing; they can be explained more readily on the assumption that the injured chromatin acts merely as a mechanical hindrance in those instances in which it is actually caught in the cleavage spindle. It is difficult to imagine how disintegrating chromatin can communicate its injury to sound chromatin. The idea is apparently drawn from O. Hertwig's theory that the radiation produces a "contagium vivum" which acts in a manner similar to the poison elaborated by a pathogenic bacterium. Such an assumption has no basis in fact and does not explain the observed phenomena.

The cytological studies of Paula Hertwig on the eggs of *Triton* and the frog, and of Opperman on the trout, indicate that whatever effect the radiated chromatin produces in the division of the unirradiated nucleus is due to mechanical interference. Such effects can best be seen when the radiated and sound nuclei actually fuse prior to the formation of the chromosomes. The radiated chromatin is eliminated from the spindle and causes a derangement in the spindle if it lies near enough to it. In *Chaetopterus* the chromosomes form before the fusion of the nuclei and there is no evidence that any such interference occurs.

There is always a noticeable variation in the extent of injury produced by the radiations on a single lot of eggs. The curves shown in the text figure indicate that after the critical length of exposure, when the egg nucleus is greatly injured in the majority of cases, there are still some eggs which develop normally with the diploid number of chromosomes. This variation may be due to the fact that the eggs were not equally exposed, or that some are more resistant than others. The latter is probably the case. Paula Hertwig ('16) showed that when *Triton* eggs are exposed for a definite period some develop normally and some abnormally. The normal eggs in certain instances showed the

diploid number of chromosomes. Her explanation for this phenomenon is that the normal sperm chromosomes divided to form the diploid number at some time before the first cleavage. There is no evidence to prove this point. More probably the egg was not radiated sufficiently and the nucleus was comparatively uninjured so that it fused with the sperm nucleus and the subsequent cleavage was normal.

That sperms show varying resistance was shown by Gunther and Paula Hertwig ('13). When methylene blue is allowed to act



TEXT FIGURE 1.

for 18 hours on sperm from the same testis, some of the sperms are killed while others retain their motility perfectly. The same variation in susceptibility is probably found in the egg also.

Signs of injury in the chromatin do not appear until the chromatin increases in volume. The radiated sperm appears perfectly normal until it commences to grow inside of the egg. In the case of the sea-urchin it normally does not increase greatly in size until after it has fused with the egg nucleus, and it is not until that time that a radiated nucleus shows its abnormal condition. When the eggs of *Chætopterus* are radiated the chromosomes are in the form of tetrads, the two successive divisions of the chromatin having already taken place. The chromosomes divide regularly and do not increase noticeably in size. Polar body formation is

normal. It is abnormal only when the egg has been radiated so long that the protoplasm has been injured. But if the radiation is for periods up to 50 minutes, the polar bodies are well formed and the remaining chromosomes appear to be normal until they grow into the vesicular condition. The altered mode of development in such cases has already been described. If the radiation is short, that is, from 20 to 30 minutes, the chromatin does not show evidences of injury until the time of cleavage. Since chromatin grows by taking up substances from the protoplasm it is evident that the radiations have acted so as to interrupt this process, probably by altering the chemical constitution of the chromatin.

The unusual movement of the nucleus in radiated eggs calls for further comment. After short exposures the nucleus moves in a normal manner to a point slightly above the center of the egg where it fuses with the sperm nucleus. But as the duration of the exposure is lengthened it shows a greater and greater tendency to remain at the pole. In every case it makes some attempt to migrate inward, but its freedom of movement appears to be inhibited by the peculiar attachment to the second polar body. In many instances, especially in eggs radiated for 35 minutes or more, this curious attachment is not found, the nucleus lying free in the protoplasm. Following the extrusion of the second polar body, a process which is normal in all respects, the remaining chromosomes become vesicular and finally fuse to form a single large vesicle, which, instead of moving toward the sperm nucleus, remains in place. The chromatin which in the course of normal development condenses into chromosomes after the two nuclei have fused here develops into irregular masses, some of which may migrate slightly; the greater number do not. That chromatin which moves furthest from the pole is most normal in appearance.

Why does the egg nucleus fail to travel toward the center of the egg? The egg protoplasm has not been visibly injured; indeed it appears to be perfectly normal for it can draw in the sperm and the latter moves at the usual rate up to the center of the egg. In both monospermic and polyspermic eggs there is evidence that extensive protoplasmic movements are taking place for the yolk

spheres are pushed away from the advancing sperm so that the latter lies in an area of clear protoplasm. In respect to the sperm, therefore, the egg protoplasm reacts normally. Only the egg nucleus fails to migrate. As the duration of exposure increases the nucleus moves a shorter and shorter distance. In other words, the greater the injury to the chromatin, the smaller is the distance through which it moves. This is shown in Fig. 11. The egg nucleus under the polar bodies has formed into chromosomes and chromatin masses, of which the most abnormal in appearance is still in its original position while the rest have moved down to a position normally occupied by the egg nucleus at this stage. This condition suggests that the chromatin is concerned in its own movement.

This idea is supported by the behavior of the radiated chromatin shown in Figs. 8 and 9. In Fig. 8 the radiated chromatin in the form of chromosomes is left behind during the division of the sperm nucleus. One chromosome is normal in appearance but the spindle fiber attachment is abnormal; instead of being median it is terminal. In this instance there is an ineffectual attempt at movement. In Fig. 9 no spindle fibers are found attached to the irregular masses of egg chromatin, and the masses do not move with the sperm chromosomes.

According to Chambers ('17) the spindle fibers are lines of stress or of protoplasmic flow; they are not formed structures. As such they are probably produced as a result of the interaction of the chromosomes on the egg protoplasm. When the chromatin is rendered abnormal by the radiations it fails to stimulate their production and therefore does not move with the sperm chromosomes to which spindle fibers are attached. If the fibers develop under the influence of the chromosomes, there is no need to assume the presence of contractile fibers in the protoplasm which serve to pull the chromosomes toward the poles. They are not bodies which are passively pulled about in the cell, but are actively concerned in their own movement.

SUMMARY.

1. Radiation of the unfertilized eggs of *Chætopterus* results in injurious effects which do not become manifest until after the

extrusion of the polar bodies, following insemination with normal sperm. That two normal mitotic divisions should intervene after radiation and only then that the injury to the chromosomes becomes apparent can be explained by the fact that the two longitudinal divisions preparatory to polar-body formation have been completed. Only when the chromosomes again "grow" in preparation for a new division do abnormal characters manifest themselves.

2. If the radiation is brief (20 to 30 min.) the germ nuclei fuse normally but the radiated egg chromatin fails to form normal chromosomes. No spindle fibers develop from these abnormal chromatin masses, and they remain in the cleavage plane during mitosis. If the radiation is longer (35 to 50 min.) the chromosomes, after the extrusion of the polar bodies, develop abnormally and fail to fuse with the sperm nucleus. In both cases the sperm nucleus divides and development is androgenetic.

3. During cleavage the most injured chromatin does not move and lacks spindle fibers. This suggests that the fibers are normally developed under the influence of the chromosomes and that the latter are concerned in their own movement.

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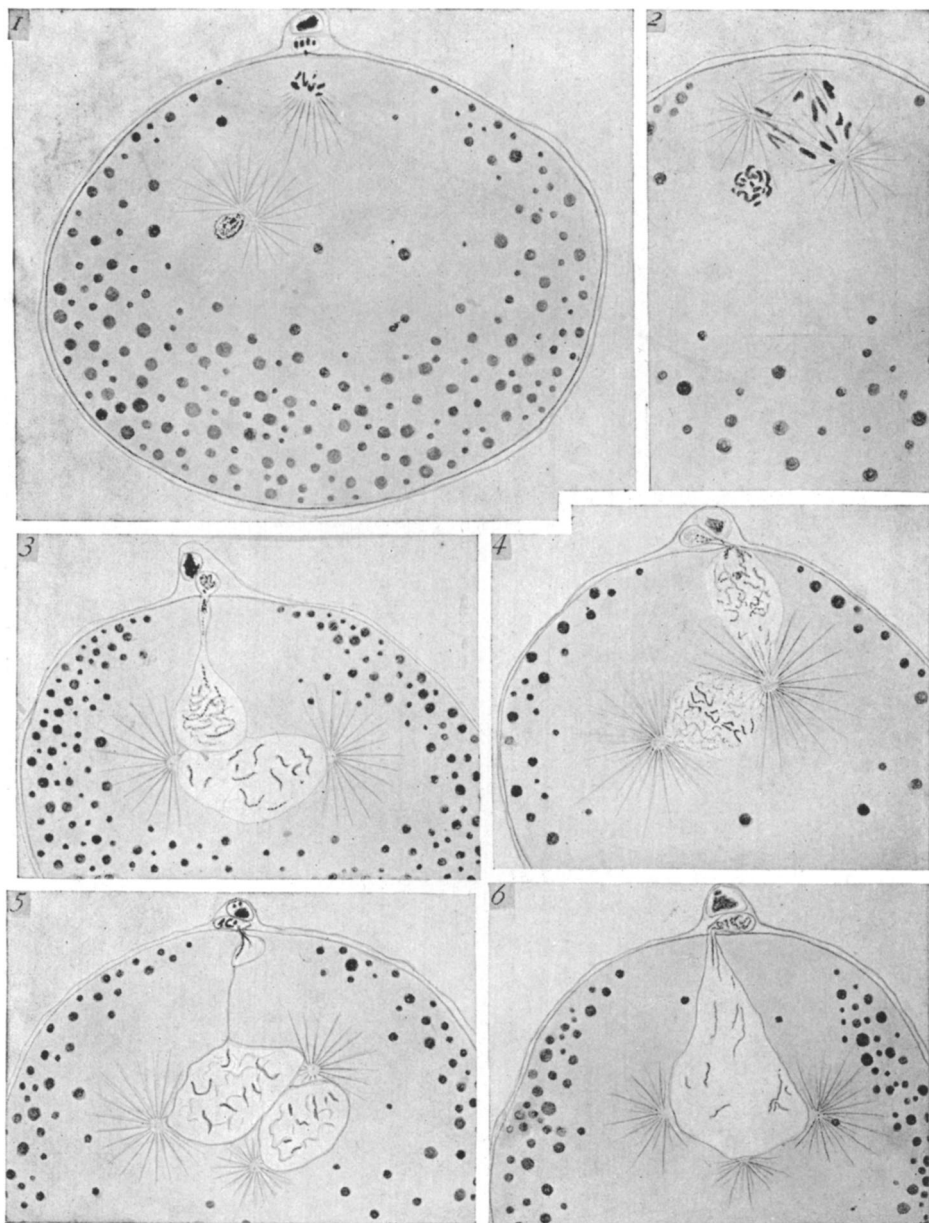
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EXPLANATION OF PLATES.

The drawings were made with the aid of a camera lucida. The lenses employed were a 1.5 mm. Zeiss Apochromatic and a No. 6 Compensating ocular.

EXPLANATION OF PLATE I.

- FIG. 1. Normal fertilization. 25 min. after insemination.
 FIG. 2. Egg radiated for 80 min. The development of the sperm is abnormal.
 FIG. 3. Egg radiated 35 min. Killed 34 min. after insemination.
 FIG. 4. " " " " " 30 min. " "
 FIG. 5. " " " " " 39 min. " "
 FIG. 6. " " " " " 39 min. " "



EXPLANATION OF PLATE II.

FIG. 7.	Egg radiated 35 min.	Killed 34 min. after insemination.		
FIG. 8.	Egg radiated 25 min.	" 42 min.	"	"
FIG. 9.	Egg radiated 35 min.	" 39 min.	"	"
FIG. 10.	" " " "	" 34 min.	"	"
FIG. 11.	" " " "	" 40 min.	"	"

